Glutaraldehyde-Crosslinked Chitosan Beads for Controlled Release of Diclofenac Sodium

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ABSTRACT: An inexpensive and simple method was adopted for the preparation of chitosan beads, crosslinked with glutaraldehyde (GA), for the controlled release of diclofenac sodium (DS). The beads were prepared by varying the experimental conditions such as pH, temperature, and extent of crosslinking. The absence of any chemical interaction among drug, polymer, and the crosslinking agent was confirmed by FTIR and thermal analysis. The beads were characterized by microscopy, which indicated that the particles were in the size range of 500–700 μm and SEM studies revealed smooth surface and spherical shape of beads. The beads produced at higher temperature and extended exposure to GA exhibited lower drug content, whereas increased drug loading resulted in enhanced drug release. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 211–217, 2007

Key words: drug delivery; controlled release; chitosan; crosslinking

INTRODUCTION

Over the past few decades, a lot of attention has been focused on the development of novel drug delivery systems for both oral and systemic applications, using various types of polymers.[1–5] Many controlled release drug delivery devices have been developed in response to changes in environmental conditions, e.g., temperature, pH, electric field, and presence of certain chemicals,[6] using a variety of techniques.[7–9] In recent years, biodegradable and biocompatible polymers have attracted a considerable attention as potential carriers for the controlled and site-specific delivery of drugs. Chitosan is a cationic, biocompatible, and biodegradable polymer having many biomedical applications. Several researchers have noted hydrogel structures, but crosslinked chitosan beads for a controlled release formulation is a novel approach reported to date.[10–14]

Chitosan is a poly(aminosaccharide), normally obtained by alkaline deacetylation of chitin, the principal component of living organisms such as fungi and crustacea. This naturally occurring polymer has a repeating unit of 2-acetamido-2-deoxy-β-D-glucose.[15] The remarkable properties of chitosan, such as its nontoxicity, solubility in dilute acids forming polyelectrolyte solutions (cationic), and complexation with anions, favor its utilization in pharmaceutical and medical fields.[16]

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Diclofenac sodium (DS) is widely used in the treatment of chronic inflammatory diseases. Earlier reports suggest that DS produces side effects such as ulceration, bleeding, or perforations of intestinal wall.[11] To increase the bioavailability and decrease the gastric irritation, suppositories, ointments, eye lotions, spray formulations, and gel preparations for topical application containing DS were prepared.[17,18]

The advantages of controlled release formulations containing the nonsteroidal anti-inflammatory agents (NSAIDs) over their conventional dosage forms have been reported. Such formulations minimize the serious gastric irritant side effects of the conventional NSAID preparations and also will reduce the dose as well as frequency of administration and hence, increase the patient compliance, which is paralleled by a reduction in health care costs and better disease management.[19]

In view of the side effects and short biological half-life, DS was developed as a controlled release formulation using chitosan. Glutaraldehyde, which is a bifunctional agent, was used as a crosslinking agent. Factors like extent of crosslinking, % loading of drug, and effect of temperature on crosslinking of polymer were studied to optimize the formulation conditions.

EXPERIMENTAL

Materials

The gift sample of diclofenac sodium (DS) was supplied by Sun Pharma, Baroda, India and used as received. Chitosan (degree of deacetylation was 86.8%) was purchased from Aldrich Chemical. Analytical grade samples of glutaraldehyde (GA) and acetic acid

were purchased from s.d. fiNE-CHEM, Mumbai, India and used as received.

Methods

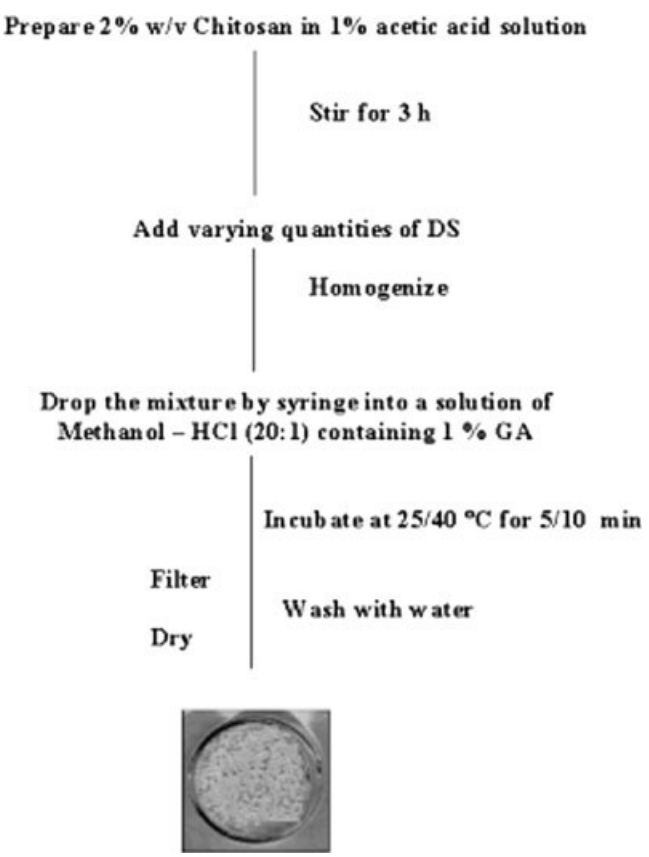
Preparation of GA crosslinked chitosan beads

A 2% w/v chitosan solution was prepared in 1% acetic acid solution and stirred for 3 h. After complete solubilization, varying quantities (30, 40, 50, and 60% w/w of dry weight of polymer) of DS was added to the above solution and mixed homogeneously. The polymer solution containing DS was added drop wise into methanol–1*N* HCl solution (20:1) containing 1% GA, using a hypodermic syringe having 1-mm diameter. The chemically reacted crosslinked beads thus resulted were removed by filtration from the mixture, washed with water, and allowed to dry in an oven (Scheme 1).[15]

The bead formation was optimized by carrying out the experiments at two different temperatures (25 and 40°C) and different exposure time to GA (5 and 10 min). Experimental conditions such as distance between syringe and water level, and number of drops per minute were maintained.

Bead size measurement

Five samples of the completely dried beads from different formulations were selected and their sizes were measured using a micrometer screw gauge (Sargent, USA) within an accuracy of ±0.01 mm.



Scheme 1 Preparation of GA-crosslinked chitosan beads.

Swelling studies

Swelling property of the beads was studied by a measurement of percentage water uptake as a function of time. Five different beads exposed to GA for different time intervals and different temperatures were selected for the study. The beads with a known weight were placed on a watch glass containing distilled water in an incubator. At regular intervals of time, the swollen beads were removed from the watch glass, blotted with the filter paper (to remove any adsorbed water on the surface), and weighed to calculate the average value.[20] The beads were weighed until a constant weight was achieved, indicating complete equilibrium. During this process, care should be taken while handling the swollen beads so as to avoid any weight loss due to breaking or erosion of the beads. All the mass measurements were made on a Mettler single pan balance having an accuracy upto fifth decimal. The percent equilibrium water uptake was calculated as

$$\frac{\text{Weight of swollen beads}\,(w_1) - \text{Weight of dry beads}\,(w_2)}{\text{Weight of dry beads}\,(w_2)} \times 100$$

Rate of drying

A few samples of the beads were selected randomly for the study and allowed to dry in an oven maintained at 40°C. Initial masses of the beads should be nearly equal for easy comparison. The beads were weighed at definite intervals of time until a constant weight was achieved.[21] To maintain the accuracy, experiments were carried out in triplicate and the average values were used for the calculation.

Drug content

Beads were evaluated for the drug content and this was done by incubating a known mass of beads with 5 mL of water. The swollen beads were crushed in mortar with a pestle and the solution thus formed was sonicated (Ikasonic U50, Ika Labortek, Germany) for 2 min using 60 MHz of frequency. Water was evaporated to form a thick paste, to which about 10 mL of methanol was added to extract the entire drug. The precipitated polymer was separated from methanol by centrifugation (Remi R24, India) for 5 min at 10,000-rpm speed. Then the absorbance of methanol containing the drug was determined using UV spectrophotometer at 284 nm and pure methanol was used as a blank.[22]

In vitro drug release studies

In vitro dissolution of beads was carried out using the dissolution apparatus (Dissotest, Lab India) equipped with six paddles. The dissolution rates were measured at 37°C under 100 rpm paddle speed. Weighed quantities of each sample were placed in buffer solutions. A 900 mL of 0.1*N* HCl was used during the first 3 h and then the medium was drained off and replaced with phosphate buffer solution (pH 7.4) to simulate the gastrointestinal (GIT) conditions. A 10 mL of aliquot was taken from the vessel at definite intervals of time and replaced with an equal volume of corresponding dissolution medium. If necessary, the samples were diluted before the assay. These samples were analyzed using UV spectrophotometer at 284 nm and concentration of DS was calculated using the calibration curves constructed from the reference standards.[23] The *in vitro* release studies were performed in triplicate for each of the samples.

Stability studies

Different samples of beads were placed in screw-capped glass containers and stored under ambient humidity conditions at different temperatures such as 60°C, 37°C, room temperature, and 5°C for a period of 3 months. The samples were checked for any physical changes and analyzed for the drug content at regular intervals of time.[24]

The sample to be tested for drug content was dipped in 5 mL of water/suitable solvent for 4 h, to extract the drug completely from the polymer. Solvent was evaporated to a dry residue in a rotary flash evaporator. The IR spectrum of this residue was compared with that of the reference standard drug to confirm the intactness of drug molecule in the formulation.

Scanning electron microscopy

The topography of beads was observed by scanning electron microscopy (SEM). The sample was deposited on a brass hold and sputtered with gold. SEM photographs were taken with JSM 6400 Scanning Microscope (Japan) at the required magnification. The working distance of 39 mm was maintained and acceleration voltage used was 5 kV, with the secondary electron image (SEI) as a detector.

Fourier transform infrared spectral studies

All the FTIR spectral data were taken on a Nicolet (Model Impact 410, USA) to find out the chemical stability of drug in the formulation. About 2 mg of the samples were ground with spectroscopic grade KBr and the pellets were prepared under a hydraulic pressure of 600 kg/cm^2. The spectra were obtained by eliminating the background noise and scanning was done in the range of 4000–500 cm^{-1}. The instrument uses a He–Ne laser (632.8 nm) as a equipment carrier with deuterated triglycine sulfate (DTGS) detector.

Thermal analysis

Differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA) were performed on different samples to determine their composition and predict the thermal stability. DSC measurements were done on a DuPont-2000 microcalorimeter (made in USA) and samples were heated at the rate of 10°C/min. TGA measurements were done on a Mettler Toledo TGA unit (Switzerland) and samples were heated upto 400°C.

RESULTS

Characterization of beads

A simple and an inexpensive method was adopted for the preparation of chitosan beads for the controlled release of DS. The beads were prepared by crosslinking of chitosan with GA in presence of methanol and HCl. Free amino groups of chitosan were reacted with GA to form a crosslinked polymeric matrix, in which DS was embedded to provide a controlled release action.

FTIR spectra of pure DS (a), chitosan (b), GA-crosslinked chitosan (c), and DS-loaded GA-crosslinked chitosan (d) were compared as shown in Figure 1. In case of pure chitosan, the characteristic band at 3425 cm^{-1} was observed and was due to O—H stretching. Amino groups of chitosan were converted into imine group (C=N), when treated with GA. A sharp peak

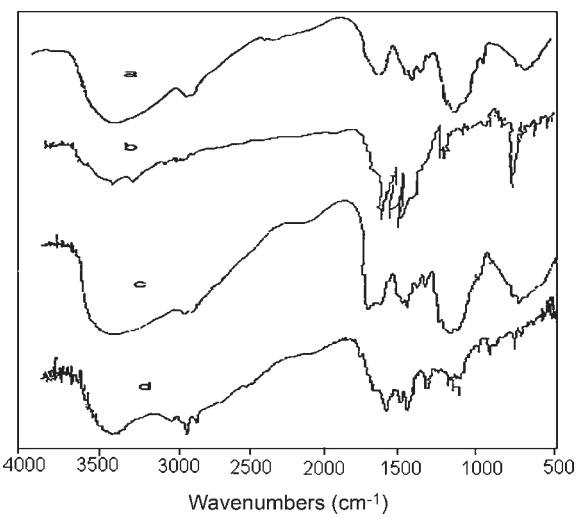


Figure 1 FTIR Spectra of (a) pure chitosan, (b) pure DS, (c) GA-crosslinked chitosan, and (d) DS-loaded GA-crosslinked chitosan.

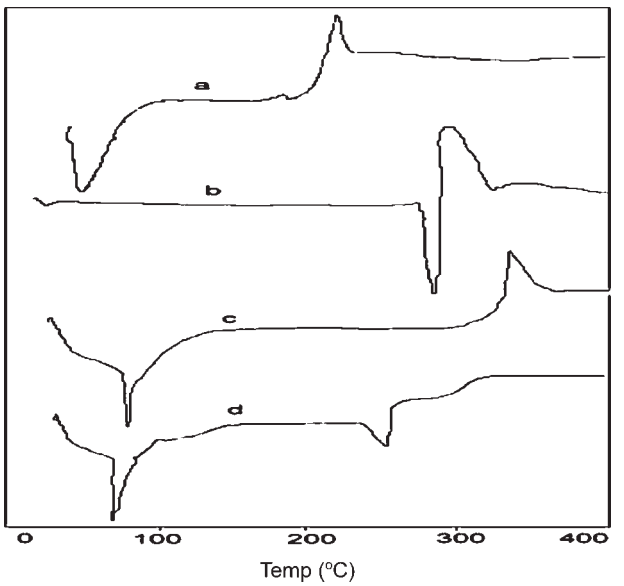


Figure 2 DSC thermograms of (a) pure chitosan, (b) pure DS, (c) GA-crosslinked chitosan, and (d) DS-loaded GA-crosslinked chitosan.

observed at 1640 cm^{-1} was due to the presence of imine group (C=N). FTIR spectra of DS showed a peak at 3079 cm^{-1}due to N—H stretching. The peaks observed at 1579 and 1455 cm^{-1} were due to C=C aromatic stretching. A sharp peak observed at 707 cm^{-1} was due to —Cl group attached to the aromatic moiety. These data are in conformity with the earlier report.[25]

When the drug was incorporated into GA-crosslinked chitosan, along with all the characteristic peaks of the crosslinked chitosan, additional bands have appeared due to the presence of DS. The FTIR spectra showed principal peaks at 3036 cm^{-1} due to N—H stretching and at 690 cm^{-1} due to C—Cl. The peak observed at 1449 cm^{-1} was due to C=C aromatic stretching. No extra peaks were seen, indicating there was no interaction of DS either with chitosan or with GA.

The DSC thermograms of pure DS (a), chitosan (b), GA-crosslinked chitosan (c), and DS-loaded crosslinked-chitosan (d) were obtained (Fig. 2).

After reacting with GA, the endothermic peak of chitosan has shifted from 50 to 68.7°C, thus making the polymer matrix more rigid, indicating the crosslinking of polymer with GA. The drug DS showed a sharp peak at 287.5°C due to its melting point, which did not appear in the DSC thermogram of DS-loaded GA-crosslinked chitosan beads. The endothermic peaks responsible for the drug clearly indicated its inactness in the formulation. However, the drug-loaded chitosan beads have shown higher T_g than pure chitosan. The significant change in T_g may be due to physical and morphological changes of beads after drug loading.

The thermal decomposition of DS (a), chitosan (b), GA-crosslinked chitosan (c), and DS-loaded GA-crosslinked chitosan (d) was studied by thermogravimetry (Fig. 3). The TGA curves and the corresponding data indicate that the compounds decompose in two steps. GA-crosslinked chitosan showed a lesser weight loss (55%) as compared to pure chitosan (60%) at the same temperature indicating, the crosslinking decreases the rate of degradation and increases the stability. The first step involves a smaller weight loss, may due to the initial loss of water molecules and loss of few fragments attached at the periphery of crosslinked chitosan. The destruction of chitosan moiety occurs in the second step.

DS undergoes decomposition at 280°C, and above 300°C, only 1% of char residue remains. The DS-loaded beads showed decomposition at the same temperature as that of pure drug and showed more weight loss (61.5%) as compared to pure chitosan. Since the melting point of drug was not altered, it

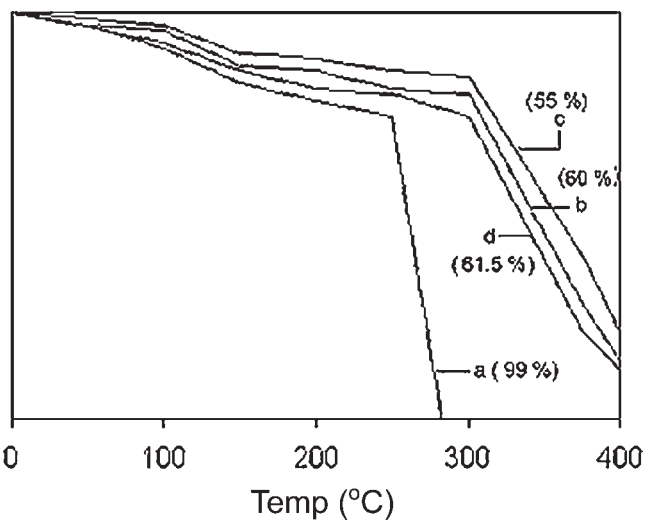


Figure 3 TGA thermograms of (a) pure chitosan, (b) pure DS, (c) GA-crosslinked chitosan, and (d) DS-loaded GA-crosslinked chitosan.

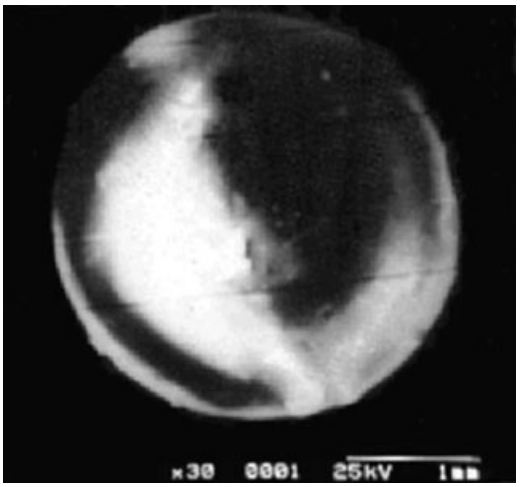


Figure 4 SEM view of GA-crosslinked chitosan bead.

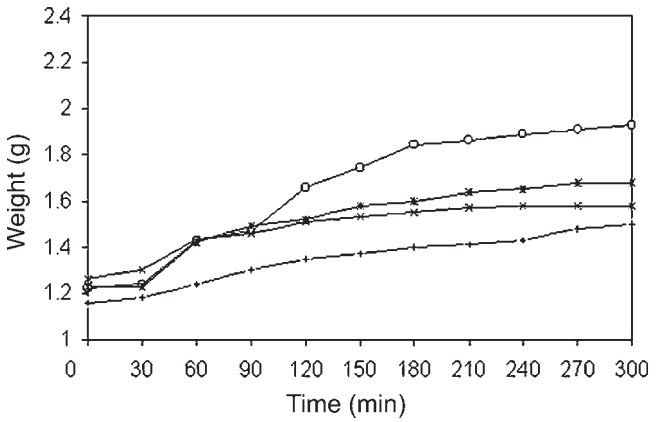


Figure 5 Rate of water uptake by beads prepared at different temperatures of methanol and different exposure times to GA: (○) for 5 min at 25°C; (*) for 10 min at 25°C; (×) for 5 min at 40°C; (+) for 10 min at 40°C.

indicates the absence of any chemical interaction of DS either with chitosan or with GA. These results further indicate that the drug-loaded beads follow a very systematic decomposition pattern when compared to either the pure drug or the polymer.

The microscopic observations revealed that the GA-crosslinked chitosan beads were in the size range of 500–700 μm. The beads were further subjected to SEM analysis, as shown in Figure 4. They were found to be spherical in shape with smooth surface. It has been observed that the particle size did not vary significantly either by increasing the exposure time to GA or by varying the amount of the drug.

Swelling studies

Swelling property of the beads has been studied by a measurement of water uptake at a particular time interval. The results of water uptake by the beads are displayed in Figure 5, which suggest that the beads absorb maximum amount of water during the first hour. The beads prepared by exposing to GA for lesser time (5 min), absorbed more amount of water than those prepared by extended exposure time (10 min). Water uptake was also dependent upon the temperature. Beads formed at higher temperature (40°C) absorbed less amount of water than those formed at lower temperature (25°C).

The drug is dispersed in almost spherical-shaped beads. Therefore, it is possible to model the diffusion process, which involves immersion of polymeric beads into the medium of interest and thereby provoking the process of absorption of liquid by the polymer. Mathematical models have been built to describe the process of absorption and desorption.[26] The diffusion coefficient (D) for water absorption can be calculated using the following equation

$$D = \left(\frac{r\theta}{6M_\infty}\pi\right)^2$$

where θ is slope of the linear portion of the plot of M_t/M_∞ versus $t_{1/2}$, r is radius of the beads, M_t is the fractional amount of solvent absorbed, and M_∞ is the maximum value for sorption.

The diffusion coefficient (D) values show dependence upon the extent of crosslinking in terms of time of exposure to GA and temperature, but not significantly on the pH. The results are given in Table I.

Rate of drying

To optimize the drying conditions, some of the beads with different process variables were selected with approximately equal initial mass. Figure 6 shows the results of rate of drying, which indicate that both the time of exposure to GA and the temperature influence the drying rate. The beads exposed to GA for longer time required excess drying time than those exposed to GA for shorter period of time. On the other hand, for the beads prepared at higher temperature, time of drying was more than those beads prepared at lower temperature.

For calculating diffusion coefficient (D) from desorption experiments, the slope θ was calculated from linear plot of

$$\ln 1 - \frac{M_t}{M_\infty} \quad \text{vs time } t.$$

TABLE I
Percentage Drug Content of GA-Crosslinked DS (50%)-Loaded Chitosan Beads

Time of exposure to GA (min)	Temperature of methanol (°C)	% HCl in methanol (v/v)	% Drug content	$D_{Sorption}$ (cm^2/s)	$D_{Desorption}$ (10^{-8}) (cm^2/s)
5	25	0.1	43.07 ± 0.14	5.73×10^{-7}	5.11
5	25	0.5	48.58 ± 0.30	5.29×10^{-7}	4.49
5	40	0.1	28.17 ± 0.41	4.78×10^{-7}	3.39
5	40	0.5	37.51 ± 0.51	4.34×10^{-7}	3.10
5	25	1.0	72.11 ± 0.24	5.81×10^{-7}	5.04
10	25	1.0	70.32 ± 0.54	5.02×10^{-7}	4.8
5	40	1.0	68.14 ± 0.34	4.19×10^{-7}	3.78
10	40	1.0	57.27 ± 0.19	7.86×10^{-8}	2.02

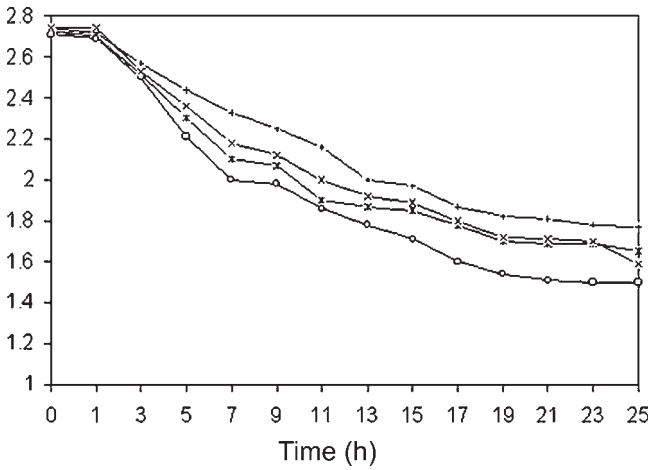


Figure 6 Rate of drying of beads prepared at different temperatures of methanol and different exposure times to GA: (○) for 5 min at 25°C; (*) for 10 min at 25°C; (×) for 5 min at 40°C; (+) for 10 min at 40°C.

It is observed that the *D* values for sorption are higher than those observed for desorption by several orders of magnitude, which is attributed to the slow drying rate of beads. However, the *D* values for desorption did not show any systematic dependence on the type of matrix. In majority of cases, the lower *D* values are observed for the matrix produced at high temperature with longer exposure time than for those produced at low temperature with lesser exposure time to GA.

Drug content

The results of drug content are compiled in Table I. It has been observed that the content of drug decreased drastically with an increase in pH. Beads prepared at lowest pH showed highest drug content (72.11%) and the lowest drug content (28.17%) was observed for the beads prepared at highest pH. DS, being a salt of weak acid, is insoluble in acidic media and hence, an increase in drug content was observed with an increase in % HCl content in methanol. Increased exposure to GA and high processing temperatures led to lowered drug content.

In vitro drug release studies

Kinetics of DS release is depicted in Figure 7. To investigate the effect of crosslinking on the release rate, the beads exposed to GA for different time intervals (5 and 10 min), at different temperatures (25 and 40°C) and loaded with 50% DS were selected. The time selected was between 5 and 10 min because less than 5 min, the beads formed were not hardened, whereas more than 10 min, the % drug content was very less due to the leaching of drug by slow solubilization. The beads prepared at 25°C and exposed to GA only for 5 min showed drug release in 90 min, whereas those prepared at 40°C and with 10 min exposure to GA released about 80% of drug after 4 h.

Release of drug from the polymer matrix is a function of extent of crosslinking. The effect of release rate of drug from the beads loaded with different quantities of drug and prepared at 25°C (exposed to GA for 10 min) is shown in Figure 8. Release rate increased with higher extent of drug loading. The release rate of 60% DS-loaded beads showed drug release in 270 min, whereas 30% drug-loaded beads showed only 70% drug release after 300 min.

Stability of beads

The beads were observed for any change in color or appearance and drug content during storage. The

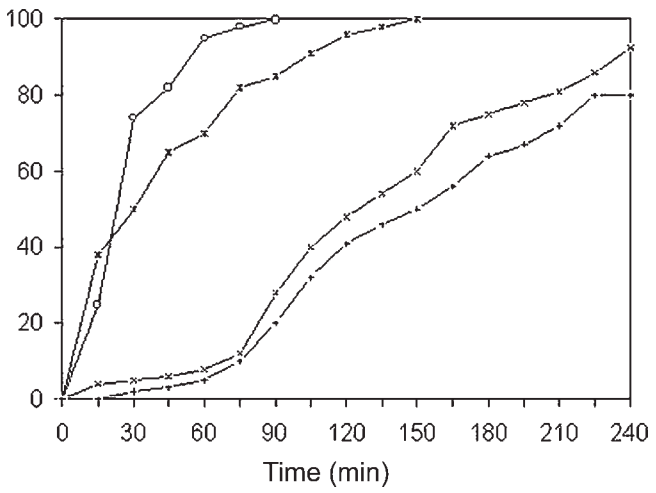


Figure 7 Amount of drug released from beads prepared at different temperatures of methanol and different exposure times to GA: (○) for 5 min at 25°C; (*) for 10 min at 25°C; (×) for 5 min at 40°C; (+) for 10 min at 40°C.

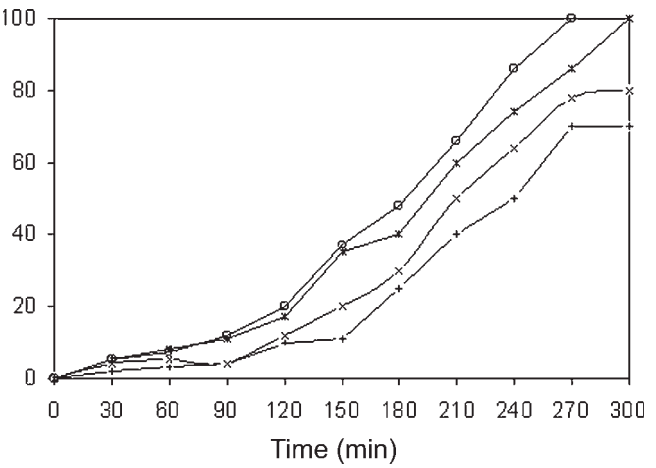


Figure 8 Amount of drug released from beads loaded with different concentrations of DS: (○) 60%; (*) 50%; (×) 40%; (+) 30%.

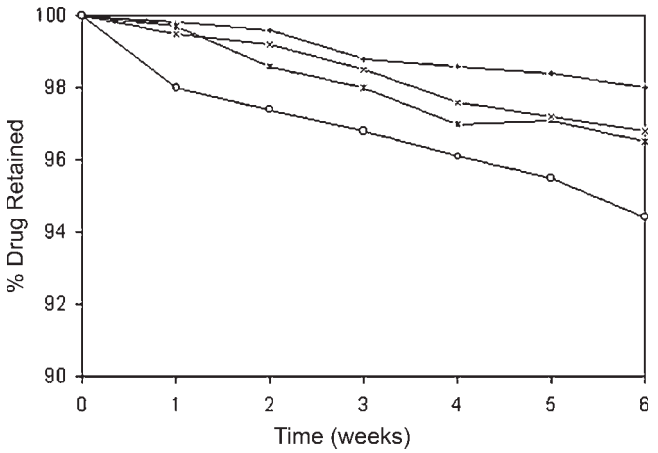


Figure 9 Relative stability of 50% DS-loaded GA-crosslinked chitosan beads at different temperatures: (○) 60°C; (*) 37°C; (×) 27°C; (+) 5°C.

effect of temperature on stability of beads is shown in Figure 9. Beads were found to be stable at all the different temperatures of study without any physical changes.

DISCUSSION

Chitosan is a hydrophilic polymer. Transport of water through the polymer depends upon the rigidity and extent of its crosslinking ability. The increased porosity and decreased crystallinity of the polymer enhance the water uptake and hence swelling ability. Crosslinking occurs much faster at high temperatures and highly crosslinked beads absorb very small amount of water.

Drug content is the amount of drug entrapped within the polymer matrix with respect to the total drug introduced into it. The drug content in a controlled release formulation reflects the space available within the matrix.

Release of DS from the beads can be influenced by a number of physicochemical parameters including those related to the pH, temperature, rate of stirring, and characteristics of the controlled release drug delivery devices (beads). The effect of temperature was more prominent than the effect of exposure time for the drug release. From the graph, it is evident that the rate of release of drug is low for the beads formed at higher temperature compared to those formed at lower temperature under the same exposure time to GA. By crosslinking the polymer, the overall matrix becomes denser so that the rate of diffusion of the drug decreases.

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